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REMARKS

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Claims 11, 15, 16, and 21-26 are now of record in the case. Claims 11, 16, 21, 22, and 25 have been amended, claims 12-14 and 17-20 have been cancelled, and new claim 26 has been added. Non-elected claims 1-10 were cancelled previously.

Support for the amendments to the claims is inherent in the original disclosure. Specifically, with respect to the additional components recited in claim 1, support for the termite aggregation attractant may be found in original claim 16.

Support for the recitation of a termite pheromone may be found in previously presented claim 25 and in the specification at paragraph no. 0031 on page 11 of the specification. Support for the recitation of a bait matrix comprising a cellulose-containing material, may be found in original claim 21 and previously presented claim 22, and in the specification at paragraph no. 0029 bridging pages 9 and 10. The recitation in claim 1 that the *Paecilomyces* comprises spores may be found in original claim 14 and paragraph no. 0027 bridging pages 8 and 9 of the specification. The recitation in claim 1 that the *Paecilomyces* comprises *P. fumosoroseus* or *P. javanicus* may be found in original claims 12 and 13, and in the specification at paragraph no. 0024 bridging pages 7 and 8. Support for the amendment to claim 21 that the bait matrix comprises a cellulose containing material may be found in previously presented claim 22, and in

the specification at lines 12-14 of paragraph no. 0029 bridging pages 9 and 10. The recitation of the various cellulose-containing materials recited in claim 22 is supported by the specification at lines 13-20 of paragraph no. 0029 bridging pages 9 and 10. Finally, support for new claim 26 reciting that the bait matrix may be a solid, semi-solid, or liquid may be found at lines 3-5 of paragraph no. 0029 bridging pages 9 and 10.

Rejection Under 35 U.S.C. §102

Claims 11, 12, 14, 15, and 17-21 have been rejected under 35 U.S.C. 102(b) as being anticipated by Osborne. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Osborne discloses methods and compositions for controlling whiteflies using the fungus *Paecilomyces fumosoroseus*. Spores of the fungus are formulated in water with a wetting agent and an optional powder or granular carrier (col. 4, line 63 to col. 5, line 30).

The instant invention is drawn to compositions for control of termites. Applicants have discovered that strains of the entomopathogenic fungus of the genus *Paecilomyces* are useful for control of infestations by subterranean termites. Spores of *P. fumosoroseus* or the closely related *P. javanicus* are used in a preferred embodiment (paragraph nos. 0024 and 0027). In another

preferred embodiment, the fungus is formulated in a bait matrix which contains a form of cellulose (paragraph no. 0029). The fungus may also be formulated with optional attractants such as a termite pheromone or a termite aggregation attractant (paragraph no. 0031). This is not disclosed or suggested by Osborne.

Although Osborne does disclose using *Paecilomyces* as a biocontrol agent, the patent discloses its use against an insect, the whitefly, which is entirely unrelated to the termites disclosed and claimed in the instant invention. Moreover, because the insects are unrelated, a practitioner of ordinary skill in the art would have no motivation to use the *Paecilomyces* of Osborne against termites as claimed.

In an effort to more clearly differentiate the instant invention, applicants have amended claim 1 to limit the claimed composition to additional components which are particularly preferred for use against termites. Specifically, claim 1 has been limited to a component selected from one or more of a termite aggregation attractant, a termite pheromone, and a bait matrix comprising a cellulose-containing material. None of these components are disclosed or suggested by Osborne. Moreover, a practitioner of ordinary skill in the art would have no motivation to use any of these components, which are suited for controlling termites, in a composition for controlling whiteflies such as disclosed by Osborne.

Rejection Under 35 U.S.C. §102

Claims 11, 12, 14, 15, and 17-19 have been rejected under 35 U.S.C. 102(b) as being anticipated by Jackson. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Jackson discloses a novel technique for producing spores of *Paecilomyces fumosoroseus* which may be subsequently used as a biocontrol agent against insects such as the whitefly. The patent discloses that the spores may combined with lactose and bovine serum albumin and dried for storage (col. 11, lines 26-30), and may be formulated with water and diatomaceous earth for application against whiteflies (col. 11, lines 50-55).

As with Osborne, Jackson does not disclose or suggest using the *Paecilomyces* against termites, but rather against the whitefly, which is entirely unrelated to the termites disclosed and claimed in the instant invention. Again, because the insects are unrelated, a practitioner of ordinary skill in the art would have no motivation to use the *Paecilomyces* of Jackson against termites as claimed.

The amendments to claim 1 which were discussed in the rejection over Osborne, similarly further distinguish over Jackson. As above, the amendments limit the claimed composition to additional components which are particularly preferred for use against termites, with claim 1 being limited to a component

selected from one or more of a termite aggregation attractant, a termite pheromone, and a bait matrix comprising a cellulose-containing material. None of these components are disclosed or suggested by Jackson, and a practitioner of ordinary skill in the art would have no motivation to use any of these components, which are suited for controlling termites, in a composition for controlling whiteflies such as disclosed by Jackson.

The Rejection Under 35 U.S.C. §102(b)

Claims 11, 14-17, 21, 22, and 25 have been rejected under 35 U.S.C. §102(b) as being anticipated by Gunner et al. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Gunner et al. relates to the biological control of termites using an entomopathogenic fungus, such as *Metarhizium anisopliae* or *Beauveria bassiana* in combination with a behavioral modifier. The behavioral modifier may be a fungus that attracts termites, such as *Gloeophyllum trabeum* or its volatile products.

Alternatively, the modifier may be a fungus that is repellent to termites, such as *Metarhizium anisopliae*. The only mention of *Paecilomyces* in the disclosure is in Claims 10 and 18, wherein it is described as being an option for the entomopathogen; and in Claims 26 and 33, wherein it is described as being an option for a fungus that is both entomopathogenic and repellent. Nowhere in

the disclosure does Gunner mention either *P. fumosoroseus* or *P. javanicus*, nor is there any disclosure outside of the claims how the *Paecilomyces* should be formulated. There is no teaching or suggestion that either of these species would be entomopathogenic and/or repellent.

The instant invention was described *supra*. As amended, the claims are now limited to compositions of spores of *Paecilomyces fumosoroseus* or *P. javanicus*. Neither of these species are disclosed or suggested by Gunner. Moreover, Gunner does not disclose or suggest using spores of the fungi.

The NCBI database at:

<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>

lists 18 named species of *Paecilomyces*, one unnamed species, and 16 isolates. This constitutes a total of 35 possibilities, of which *P. fumosoroseus* and *P. javanicus* are two. Given this vast array of choices, the person of ordinary skill in the art confronted with the teachings of Gunner et al. would not have sufficient direction or motivation to select the claimed species.

Moreover, Khader Khan et al. (1993, *Insect Sci. Applic.*, 14:529-535, a copy of which is enclosed herewith), which was cited in the parent application, would further teach away from the instant invention. Specifically, Khader Khan teaches in Table 1 and at the top of page 532 that *Paecilomyces fumosoroseus* is slightly pathogenic against *Odontotermes obesus* (Rambur), an

African and South Asian termite. *O. obesus* belongs to the family Termitidae, a completely different family from the Rhinotermitidae described in Applicants' disclosure and recited in the claims presented for examination. Table 2 of the reference shows that the mortality rate of *O. obesus* treated with *P. fumosoroseus* was a mere 2% for workers major and workers minor, and 1% for soldiers. Of the nine fungal pathogens evaluated by Khader Khan, *P. fumosoroseus* was by far the worst, significantly inferior to the mortality rates as high as 80% observed for the other fungal pathogens evaluated. Based on these results, there would be absolutely no motivation for the person of ordinary skill in the art to even consider using *P. fumosoroseus* as a fungal control agent against other species of termites, particularly those belonging to a different taxonomic family than *O. obesus*. If anything, the disclosure of Khader Khan teaches away from the invention.

In contrast to the results observed by Khader Khan, Applicants demonstrate a mortality rate of 75-100% after 20 days post-exposure for the several strains of *P. fumosoroseus* evaluated in Example 1 against the Formosan subterranean termite (FST; *Coptermes formosanus* Shiraki; see FIG. 1). Two of the strains tested in Applicants' Example 1 gave a mortality rate of 100% within 6 days at an application rate of 10^9 blastospores/ml. Comparable, or even better results were reported in Applicants'

Examples 2-5 (FIGS. 2-5) for various strains of *P. fumosoroseus* against either FST or native subterranean termites (*Reticulitermes flavipes*). Example 6 shows mortality rates approaching 80% for native subterranean termites exposed to *P. javanicus*. In Example 7, *P. javanicus* achieves 100% mortality of Formosan subterranean termites in 13 days post-exposure.

Thus, the skilled artisan would be led away from using *P. fumosoroseus* in view of the negative results reported for that species as a control agent for *Odontotermes obesus*.

Rejection Under 35 U.S.C. §102(b)

Claims 11, 14-17, 21, 22, and 25 have been rejected under 35 U.S.C. §102(b) as being anticipated by Stamets. Applicants respectfully disagree and have amended the claims in an effort to more clearly differentiate over the reference.

Stamets discloses using the pre-sporulation (preconidial) mycelial stage of various entomopathogenic fungi, including *Paecilomyces* spp., for the control of a variety of insects, including termites. At numerous instances in the patent, Stamets expressly teaches the criticality of using mycelia of the fungi which are at a preconidial stage of growth which is prior to conidia or spore formation. See, for example, lines 1-5 of paragraph no. 0018 on page 2, paragraph no. 0022 on page 3, and paragraph no. 0030 on page 3.

The instant invention was described *supra*. Again, the instant claims have been limited to spores of the fungus, in accordance with the preferred embodiment of the instant invention, wherein spores of *P. fumosoroseus* or the closely related *P. javanicus* are used for control of termites (paragraph nos. 0024 and 0027). This is not disclosed or suggested by Stamets.

Stamets expressly discloses using mycelia of the fungus at a preconidial stage, before conidia or spores are formed (lines 1-5 of paragraph no. 0018 on page 2, paragraph no. 0022 on page 3, and paragraph no. 0030 on page 3). Thus, the reference would actually teach away from using spores of the fungus against termites. Indeed, the reference would also teach against using spores of any of the fungi of the other references against termites.

As noted *supra*, a copy of the publication of Khader Khan et al. has been enclosed for the Examiner's convenience. The document has not been submitted with an Information Disclosure Statement (with its accompanying fees) or listed on a form PTO-1449 because it does not: (1) establish a *prima facie* case of unpatentability, or (2) refute any position taken by applicants, as defined by 37 CFR 1.56(b).

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USDA ARS Ofc Tech Trnsfr. (309) 681-6688

P. 16

In view of the foregoing, applicants respectfully submit that claims 11, 15, 16, and 21-26 distinguish over the prior art of record. Allowance thereof is respectfully requested.

Respectfully submitted,


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enclosure:
--Khader Khan et al. (1993, Insect Sci. Applic., 14:529-535 (7 pages)

Insect Sci. Applic. Vol. 14, No. 4, pp. 529-535, 1993
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0191-9040/93 \$3.00 + 0.00
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MUSCARDINE FUNGI FOR THE BIOLOGICAL CONTROL OF AGROFORESTRY TERMITE *ODONTOTERMES OBESUS* (RAMBUR)

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(Received 2 September 1991; accepted 14 October 1992)

Abstract—Among the eight entomopathogenic fungi tested against the termite, *Odontotermes obesus* (Rambur), five fungal pathogens viz., *Beauveria bassiana* (Bals.) Vuill., *Metarrhizium anisopliae* (Metsch.) Sorokin var. *anisopliae*, *M. flavoviride* Gams Roesypal var. *minus*, *Paecilomyces lilacinus* (Thom.) Samson and *P. fumosoroseus* (Wize) Brown & Smith, were pathogenic. Three other fungal pathogens viz. *Verticillium lecanii* Zinn, *Paecilomyces farinosus* (Holm. ex Gray) and *Nomuraea rileyi* (Farlow) were not pathogenic. *B. bassiana* was the most effective pathogen among the pathogenic followed by *M. anisopliae*, *M. flavoviride*, *P. lilacinus* and *P. fumosoroseus*. Workers minor, of the three morphogenetic forms of *O. obesus*, were the most susceptible, followed by workers major and soldiers caste. Bioassay on isolates of two most virulent termite pathogens, *B. bassiana* and *M. anisopliae* revealed that *B. bassiana* isolate Bapatla was the most effective fungal pathogen with the lowest LC₅₀ (9.98×10^4 conidia/ml).

Key Words: *Odontotermes obesus*, bioassay, fungal pathogens

Résumé—Huit champignons entomopathogènes ont été testés contre les termites *Odontotermes obesus* (Rambur). Cinq espèces se sont révélées efficaces: *Beauveria bassiana* (Bals.) Vuill., *Metarrhizium anisopliae* (Metsch.) Sorokin var. *anisopliae*, *M. flavoviride* Gams Roesypal var. *minus*, *Paecilomyces lilacinus* (Thom.) Samson et *P. fumosoroseus* (Wize) Brown & Smith. *B. bassiana* et *M. anisopliae* ont été suivis par *M. anisopliae*, *M. flavoviride*, *P. lilacinus* et *P. fumosoroseus*. Les trois morphotypes de *O. obesus* testés, les petits ouvriers ont été les plus affectés, suivis par les grands ouvriers puis les soldats. Les trois autres champignons pathogènes, *Verticillium lecanii* Zinn, *Paecilomyces farinosus* (Holm. ex Gray) et *Nomuraea rileyi* (Farlow) n'ont pas donné de résultat positif. Les essais biologiques sur les isolats obtenus sur les deux entomopathogènes les plus virulents, *B. bassiana* et *M. anisopliae*, ont montré que l'isolat *B. bassiana* Bapatla a été le plus efficace avec les valeurs LC₅₀ (9.98×10^4 conidia/ml) et LT₅₀ (83,66 hr) les plus faibles.

Mots Clés: *Odontotermes obesus*, essai biologique, champignon pathogène

INTRODUCTION

Termites are an important pest of agriculture, horticulture and wood work of buildings and they are highly organized social insects. Their hidden way of life and difficulties in exactly locating the sub soil nests of subterranean termites make a direct chemical

control extremely difficult.

Search for alternatives to chemical pest control has assumed greater importance in recent years, due to the increasing cost of synthetic chemical pesticides, outbreak of secondary pests, insecticidal resistance and pollution problems. One such alternate method is biological control. Entomopathogenic fungi have been used in the past to control many pest species of economic importance, (Jayaraj, 1985, 1986). Except for the report made by Hanel (1981) no quantitative

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bioassays have been developed to compare the virulence of fungal pathogens against termites. Although Flanel (1982) and Hanel and Watson (1983) did get some encouraging results, the biological control of *Nasutitermes exitiosus* (Hill) with *Metarrizium anisopliae* Sorokin in Australia was not a permanent one. In India, except for the reports by Sermasi (1969) that *Aspergillus flavus* Link ex Gray was pathogenic to termite queen, practically no work has been done with fungal pathogens. Hence, the present study was conducted under laboratory conditions to establish the pathogenicity of different fungal pathogens and compare their virulence against termites.

MATERIALS AND METHODS

Source of termites

Termites were cultured in the laboratory on soil, soft wood and fungus garden collected from the termitearium as per Ausa et al. (1962). The cultures were maintained at $25 \pm 2^\circ\text{C}$ and 96–100% r.h. The fungal combs with termites were taken and were carefully broken on a smooth surface, and the different termite castes were separated using a fine hair brush. The three morphogenetic forms could easily be recognized (Agarwal, 1978). The workers were food gatherers and formed the bulk of the termite

population, while colony and were uniform size.

Screening assay

The fungal isolates were grown on Table 1. The isolates at 25°C were used. The conidia application by spacers using 7 (Rembach et al., 1986).

The harvests through double rpm) for 20 s solution of 0.01% Tween in the filtrates improved Net viability was (1986). Differ from 10^4 through dilutions up to 0.02% Tween.

Pathogenicity were conducted in water. T (9 cm) lined bottom directly sprayed an atomizer.

The coating Tween 80% in drying the isolates transferred to sieved (2 mm) fresh fungal culture provided as follows:

Table 1. Different fungal isolates, their source and culture medium used

| Fungal isolate | Source | Fungus further referred | Mycological culture medium used |
|--------------------------------|--|-------------------------|---|
| <i>Beauveria bassiana</i> | International Rice Research Institute (IRRI), The Philippines | PHP | Sabouraud dextrose agar enriched with 1% yeast extract (SDAY) |
| | Indian Agricultural Research Institute (IARI), New Delhi | NDL | SDAY |
| | Indian Institute of Horticultural Research (IIHR) Bangalore, Karnataka | BNG | SDAY |
| | Anamalai Hills, Coimbatore, Tamil Nadu | CBE | SDAY |
| | Agricultural Research Station Bapatla, Andhra Pradesh | BPT | SDAY |
| <i>Metarrizium anisopliae</i> | IRRI, The Philippines | Ma | Emerson's yeast phosphate solution starch agar (YPS agar) |
| <i>M. flavoviride</i> | IRRI, The Philippines | | (YPS agar) |
| <i>Paeciliomyces lilacinus</i> | IIHR, Bangalore, Karnataka | — | Potato dextrose agar (PDA) |
| <i>P. fumosoroseus</i> | Kerala Forest Research Institute (KFRRI), Peechi, Kerala | — | PDA |
| <i>P. farinosus</i> | Kerala Forest Research Institute (KFRRI), Peechi, Kerala | — | PDA |
| <i>Nanurastriatula</i> | IRRI, The Philippines | — | YPS agar |
| <i>Verticillium lecanii</i> | Tamil Nadu Agricultural University (TNAU) Coimbatore, Tamil Nadu | — | PDA |

PHP = Philippines isolate.

NDL = New Delhi isolate.

BNG = Bangalore isolate.

CBE = Coimbatore isolate.

BPT = Bapatla isolate.

Table 2.

| Fungus | Isolate |
|-----------------|---------|
| <i>M. flavo</i> | Ma |
| <i>Paecili</i> | — |
| <i>P. fum</i> | — |
| <i>B. bass</i> | PHP |
| <i>B. bass</i> | NDL |
| <i>B. bass</i> | BNG |
| <i>B. bass</i> | CBE |
| <i>B. bass</i> | BPT |

Mean

PHP = —

NDL = —

BNG = —

CBE = —

BPT = —

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population, while soldiers were the defenders of the colony and were in number. Active insects with uniform size were selected for treatment.

Screening assays

The fungal isolates obtained from different sources were grown on standard culture media as given in Table 1. The isolates which had grown for 10 days at 25°C were used for the preparation of the inoculum. The conidia were harvested freshly just before application by washing from the surface of the culture plates using 75 ml solution of 0.02% Tween 80² (Rembach et al., 1986).

The harvested conidial suspensions were filtered through double layer muslin and centrifuged (3000 rpm) for 20 min and then resuspended in 25 ml solution of 0.02% Tween 80². Conidial concentration in the filtrates was determined with the help of an improved Neubauer haemocytometer and conidial viability was determined as suggested by Gillespie (1986). Different concentrations of inoculum ranging from 10³ through 10⁵ conidia/ml were standardized by dilutions using sterile distilled water containing 0.02% Tween 80² (Rosenblatt and Vogel, 1971).

Pathogenicity tests with different fungal isolates were conducted using a uniform dose of 10⁵ conidia/ml in water. The termites were taken in a Petri-dish (9 cm) lined by filter paper (Whatman 100) and were directly sprayed with 3 ml conidial suspension using an atomizer.

The control insects were sprayed with 0.02% Tween 80² in sterile distilled water only. After air drying the treated termites they were carefully transferred to Petri-dishes (9 cm) containing fine sieved (2 mm) and sterilized soil. Small pieces of fresh fungal combs and a few soft wood pieces were provided as food. The treated insects were maintained

at 25 ± 2°C and 96–100% r.h. Insects (100) were used for each treatment and there were four replications. Observations on the mortality of termites were recorded at every 12 hr upto 7 days. To confirm the pathogenicity of the fungi the entomopathogenic fungi were isolated from the infected termites and fresh healthy termites were reinoculated.

Bioassay

Bioassay tests were carried out with five isolates of *B. bassiana* and one *M. unisporae* strain obtained from different locations (Table 1). A range of conidial concentrations from 10³ through 10⁵ conidia/ml of each of the fungal pathogen isolate were prepared and termites were treated as indicated in screening assays. Observations on the mortality were recorded at 6 hr interval for 7 days. The assay procedure was followed for all the three morphogenetic forms, namely workers major, workers minor and the soldier caste.

Statistical analysis

The mortality data were transformed to Arcsin percentage (for percentage data) or square root values for the purpose of statistical analysis, and per cent mortalities were corrected as suggested by Abbott (1925). The treatment means were separated by Duncan's multiple range test (DMRT). The data collected on dosage-mortality and time-mortality responses was subjected to "Probit analysis" (Finney, 1968).

RESULTS AND DISCUSSION

Among the fungal pathogens tested against *O. ochraceus*, all of the *B. bassiana* and *M. unisporae*

Table 2. Pathogenicity of fungal pathogens against *Orthotermes ochraceus*

| Fungus | Per cent corrected mortality after seven days | | |
|--------------------------------|---|---------------|----------|
| | Workers major | Workers minor | Soldiers |
| <i>Mycogone unisporae</i> (Mu) | 43.67d | 67.33d | 11.67ad |
| <i>M. flavoviride</i> | 8.00e | 13.33e | 5.83de |
| <i>Paeciliomyces lilacinus</i> | 6.33e | 8.00f | 3.33e |
| <i>P. fumosoroseus</i> | 2.00f | 2.83fg | 1.66ff |
| <i>B. bassiana</i> (BNG) | 54.33c | 57.67c | 31.67bc |
| <i>B. bassiana</i> (NDL) | 37.33d | 44.67d | 24.00d |
| <i>B. bassiana</i> (BPT) | 76.67a | 80.67a | 51.00a |
| <i>B. bassiana</i> (CBE) | 65.00d | 52.33cd | 36.33c |
| <i>B. bassiana</i> (PHP) | 68.67b | 70.33b | 34.67ab |

* Means separation by DMRT at 5% level for each termite caste.

PHP = Philippines isolate.

NDL = New Delhi isolate.

BNG = Bangalore isolate.

CBE = Coimbatore isolate.

BPT = Bengal isolate.

Isolates were virulent, affecting significantly higher mortalities than *M. flavoviride*, *P. illectis* and *P. fumosoroseus*, which were only slightly pathogenic. Among the five isolates of *B. bassiana* viz. CBE, NDL, PHP, BPT and BNG, the strain BPT isolated for Andhra Pradesh caused highest mortality among all the three morphogenetic forms of termites (Table 2). Workers minor were most susceptible to all the pathogens tested followed by workers major. The soldiers were the least affected by fungal pathogens (Table 2). Pathogens such as *Verticillium lecanii*, *Paecilomyces farinosus* and *Nomuraea rileyi* were not pathogenic as they neither caused mortality nor any kind of abnormality.

The probit analysis of the dosage-mortality response and time-mortality response was carried out on the three morphogenetic forms of *O. obesus* with the five isolates of *B. bassiana* and one isolate of *M. anisopliae*. Results indicated that *B. bassiana* isolate BPT was the most effective recording the lowest LC₅₀ and LT₅₀ values followed by isolates PHP, BNG, CBE, MA and NDL, respectively. It

further confirmed that workers minor was the most susceptible termite caste followed by workers major and soldiers, respectively (Tables 3 and 4).

The variation in virulence among the different isolates within the single species viz. *Bacillus bassiana* may be due to heterokaryosis, somatic recombination and saprobic growth of the fungus in the environment prior to its interaction with the insects (Roberts and Yendol, 1971). *Metarhizium anisopliae* is one of the most frequent fungal pathogens of soil insects. It attacks larvae of Tipulidae, Elateridae, Carabidae and Scarabaeidae of the genera *Hoplo*, *Amphimallon* and *Melolontha*. Epizootics have, however, been found affecting only wire worms (*Agriotes spp.*) and larvae of *Amphimallon solstitialis*. This variation in infection may be due to the presence of different pathotypes (strains) with different host preference (specificity); (Feron et al., 1972; Fargues, 1976). It is well established, now, that entomopathogenic fungi have a certain specificity (Fargues and Renaudiere, 1977). In the same species of fungus, different strains can have very different

Table 3. Susceptibility of Odontotermes obesus to different fungal isolates

| Fungus | Termite caste | χ^2 (3) | LC ₅₀ (Conidia/ml) $\times 10^3$ | Fiducial limits (95%) $\times 10^3$ |
|----------------------------|---------------|-----------------|--|--|
| <i>B. bassiana</i> (BNG) | MA | 2.09 | 32.89 | 39.38-71.01 |
| | MI | 2.09 | 36.90 | 22.92-59.41 |
| | SO | 0.24 | 350.43 | 377.79-601.97 |
| <i>B. bassiana</i> (NDL) | MA | 1.75 | 330.63 | 222.69-490.87 |
| | MI | 1.48 | 221.12 | 144.18-339.11 |
| | SO | 3.36 | 949.72 | 603.39-1489.89 |
| <i>B. bassiana</i> (BPT) | MA | 0.50 | 9.98 | 7.86-13.54 |
| | MI | 1.93 | 5.65 | 3.55-9.00 |
| | SO | 2.06 | 52.50 | 32.48-84.86 |
| <i>B. bassiana</i> (CBE) | MA | 4.14 | 148.79 | 98.20-225.44 |
| | MI | 1.83 | 114.91 | 79.29-166.54 |
| | SO | 2.72 | 690.77 | 466.57-1058.50 |
| <i>M. anisopliae</i> (PHP) | MA | 1.28 | 36.46 | 27.85-47.73 |
| | MI | 1.38 | 12.98 | 8.66-19.47 |
| | SO | 1.32 | 287.44 | 173.18-477.17 |
| <i>M. anisopliae</i> (MA) | MA | 1.05 | 225.32 | 160.21-316.89 |
| | MI | 2.08 | 148.67 | 101.17-218.48 |
| | SO | 0.40 | 780.38 | 502.17-1212.71 |

* All lines are significantly a good fit ($P < 0.05$).

MA = Workers Major.

MI = Workers Minor.

SO = Soldiers.

PHP = Philippines isolate.

NDL = New Delhi isolate.

BNG = Bangalore isolate.

CBE = Coimbatore isolate.

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15.39-1489.897.86-13.54
3.35-9.00
32.48-34.8618.20-325.44
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14.57-100.4427.45-57.73
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61.17-218.48
12.17-1212.71Table 4. Time-mortality response of *Odonotermes obesus* to different fungal isolates treated at concentrations of 4×10^7 conidia/ml.

| Fungus | Termite castes | χ^2 ^a (3) | LC_{50} (days) | Fiducial limits (95%) days |
|---------------------------|----------------|------------------------------|---------------------|-------------------------------|
| <i>B. bassiana</i> (BNG) | MA | 1.16 | 3.86 | 3.69-4.03 |
| | MI | 1.38 | 3.77 | 3.66-3.88 |
| | SO | 1.17 | 4.56 | 4.38-4.74 |
| <i>B. bassiana</i> (NDL) | MA | 1.62 | 4.62 | 4.46-4.78 |
| | MI | 1.93 | 4.45 | 4.30-4.61 |
| | SO | 1.22 | 5.89 | 3.63-6.15 |
| <i>B. bassiana</i> (BPT) | MA | 1.58 | 3.49 | 3.37-3.61 |
| | MI | 0.64 | 3.26 | 3.13-3.40 |
| | SO | 0.71 | 4.02 | 3.87-4.17 |
| <i>B. bassiana</i> (CBE) | MA | 1.66 | 4.23 | 4.10-4.36 |
| | MI | 1.53 | 3.40 | 3.75-4.23 |
| | SO | 0.80 | 4.94 | 4.78-5.08 |
| <i>B. bassiana</i> (PHP) | MA | 1.27 | 3.83 | 3.60-4.07 |
| | MI | 1.64 | 3.66 | 3.52-3.81 |
| | SO | 1.17 | 4.37 | 4.23-4.52 |
| <i>M. anisopliae</i> (MP) | MA | 2.13 | 4.50 | 4.36-4.63 |
| | MI | 1.28 | 4.05 | 3.91-4.21 |
| | SO | 0.68 | 5.83 | 5.16-5.52 |

PHP = Philippines isolate.

NDL = New Delhi isolate.

BNG = Bangalore isolate.

CBE = Coimbatore isolate.

BPT = Spain isolate.

activity spectra (Feron et al., 1972). Study of this specificity by Fargues et al. (1976) showed the importance of mechanisms acting at the integument and in the body cavity.

Silva and Bezençon (1972) found variation in toxin production in different strains of *B. bassiana*, which could be correlated with the variation in virulence. Variation in the susceptibility of insectid larvae to different geographical isolates of *Nasonia* was also observed by Igualdo et al. (1976). Feron (1978) found obvious differences in virulence between numerous strains of *B. brongniartii* against *Melolontha melolontha* L. and *Acanthoscelides obtectus* (Say). Field data suggest that strains of the same fungus exist, which are pathogenic only to adults of *M. melolontha* and others which attack only the larvae (Keller, 1986). In nature, living organisms such as the mycopathogens, undergo selection, recombination and mutation depending upon their ecological situation. This influences the genetic make up of the fungi which in turn can affect their virulence for a particular insect host (Igualdo and Gartia, 1985).

There was variation in the relative susceptibility of the different developmental stages of *O. obesus*.

The variation in the mortalities is a reflection of the progressive development of the entomopathogenic fungi over time. This phenomenon has been well documented by Gazzola (1968), Gardner and Noblet (1978), Igualdo et al. (1978) and Fargues and Rodriguez-Rueda (1980).

Variation in the susceptibility of the different forms of *O. obesus* may be as a result of cuticular or haemocoelic differences between the castes. Wood and Grula (1984) reported that the diatomaceous composition of the larval cuticle varied between instars and discussed the possible influence of amino acid combination, certain amides and peptides on the infectivity of fungal pathogens. Host-pathogen interaction also occur in the haemocoel where many intrinsic factors operate. The chemical constituents of an insect also vary as it ages (Boman, 1981).

Mechanism of pathogenicity and specificity appears to be complex and successful fungal penetration into the host can be said to depend upon at least three factors, the abilities of the spore to adhere to the cuticle, to germinate and to penetrate enzymatically (Fargues and Remaudier, 1977; Hall and Papierok, 1982). Evidently, there are many factors at the level of the cuticle and haemolymph which

may determine host specificity of fungi. A high degree of host specificity is observed in some species of termites such as *M. anisopliae* (Fargues, 1981) and *B. bassiana* (Kunimoto et al., 1977). Much more effort is needed as the subjects of infection mechanisms and host defences to elucidate the reasons for host specificity (Hall and Papiernik, 1982).

Entomopathogenic fungi viz. *B. bassiana* and *M. anisopliae* have been used, as biocontrol agents, with varying degree of success, against soil dwelling insects and termites (Keller and Zimmermann, 1989; Henel, 1981, 1982). Henel and Watson (1983) reported heavy mortalities in the laboratory colonies of *Nasutitermes exitiosus* due to *M. anisopliae* and the disease persisted for at least 15 weeks. Further, the temperature (25–30°C) and 92–96% r.h. within the mounds of *O. obesus* does not show any circadian or seasonal fluctuations (Agarwal, 1979). Thus, these two abiotic factors are in a range most suitable for maximum sporulation and conidial germination of these entomopathogens (Waleed et al., 1970; Hussey and Tinsley, 1981).

From the light of the present study and findings of Henel and Watson (1983) one gets a strong feeling that these mound building termites could be controlled using these fungi. However, there is need for further studies on the selection of fungal strains that can cope up with conditions in termite mounds. Similarly, further investigations are needed on the standardization of the dose, method of application, etc.

REFERENCES

- Abram W. S. (1923) A method of comparing the effectiveness of an insecticide. *J. Econ. Entomol.* 16, 265–267.
- Agarwal V. B. (1975) Studies on the biology and ecology of mound building termites. *Odontotermes microdentatus* Roushaw and Santschi and *Odontotermes obesus* (Rambur) Lusitan Termitidae. Ph.D. Thesis, University of Meerut.
- Agarwal V. B. (1978) Seasonal fluctuations of different castes as found in the fungus combs of *Odontotermes obesus* (Rambur) and *Odontotermes microdentatus* Roushaw and Santschi. In *Soil Biology and Ecology in India*. Edited by C. A. Edwards and G. K. Veerach, UAS, Tech Ser. 22, 192–196.
- Auer A., Chandra P. S., Koshi T., Peat S. L. and Ranganathan S. K. (1962) Laboratory culturing of termites. In *Termites in the Humid Tropics*. Proc. New Delhi Symp. 1960, 121–125 UNESCO, Paris.
- Bosman H. G. (1981) Insect responses to microbial infections. In *Microbial Control of Pest and Plant Diseases 1970–1980* (Edited by Burgess, H. D.) pp. 769–784. Academic Press, London, New York.
- Fargues J. (1976) Spécificité des chémopathogènes pathogènes importés (Hyphomycètes) pour des larves de coléoptères (Scarabacidae et chrysomélidae). *Entomophaga* 21, 313–323.
- Fargues J. (1981) Spécificité des Hyphomycètes entomopathogènes et résistance interspécifique des larves d'insectes. I. These Doctorat des Sciences naturelles, Université Paris VI.
- Fargues J. and Reauverture G. (1977) Considerations on the specificity of entomopathogenic fungi. *Mycopathologia* 62, 31–37.
- Fargues J., Robert P. H. and Vey A. (1976) Role du tegument et de la défense cellulaire des Coléoptères lors de la spécificité des vouches entomopathogènes de *Metarrhizium anisopliae*. *C. R. Acad. Sci. Series D*, 282, 2223–2226.
- Fargues J. and Rodriguez-Rueda D. (1980) Susceptibility of the larvae of *Spodoptera littoralis* (Lep: Noctuidae) to the entomopathogenic hyphomycetes *Nomuraea rileyi* and *Paeciliomyces fumosoroseus*. *Entomophaga* 25, 43–45.
- Ferron P. (1978) Étiologie et épidémiologie des mycotardes. These, Univ. P. et M. Curie, Paris, France.
- Ferron P., Hurpin B. and Robert P. H. (1972) Sur la spécificité de *Metarrhizium anisopliae* (Metsch.) Saccin. *Entomophaga* 17, 165–176.
- Finney D. J. (1954) *Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve*. Second edition. Cambridge University Press, London.
- Gardner W. A. and Noblett R. (1978) Effects of host age, route of infection and quantity of inoculum on the susceptibility of *Heliothis virescens*, *Spodoptera frugiperda* to *S. bassiana*. *J. Georgia Entomol. Soc.* 13, 210–222.
- Gezzi L. W. (1961) *Sphaerotilus rileyi* (Farlow) Charles. An ectomycorrhizal fungus of *Trichoplusia ni* (Hubner). *J. Insect Pathol.* 3, 2–10.
- Gillespie A. T. (1986) Effect of ectomycorrhizal fungi on the brown planthopper of rice *Nilaparvata lugens*. In *Biotechnology and Crop Improvement and Protection Monograph No. 34* (Edited by Day Peter R.). Proc. Symp. British Crop Protection Council, Cambridge, 264 p.
- Hall R. A. and Papiernik B. (1982) Fungi as biological control agents of arthropods of agricultural and medical importance. *Parasitology* 84, 205–240.
- Henel H. (1981) Virulence Metarrhizium the term (Isopeltis 9–10).
- Henel H. (1982) for the Nasutitermes 94, 237.
- Henel H. an field test for the e (Isoptera 303–313.
- Hussey N. W. of insect China. In Diseases pp. 783–York.
- Ignoffo C. M. Ignoffo C. M. and rala Mississippi Invertebrates 163–168.
- Ignoffo C. M. G. Thom R. (1978) bacterium Helicobitis 163–168.
- Ignoffo C. Dockers: cabbage banting leekines Hammar 262.
- Jayaram S. (1976) pest of Interpret by Jayaram India.
- Jayaram S. (1977) protectiv. 107.

Biological control of termite

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- Hanel H. (1981) A bioassay for measuring the virulence of the insect pathogenic fungus *Meteariizium anisopliae* (Metsch.) Scrolding against the termite *Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae). *Z. Angew. Entomol.* 92, 9-18.
- Hanel H. (1982) Selection of a fungus species suitable for the biological control of the termite *Nasutitermes exitiosus* (Hill). *Z. Angew. Entomol.* 94, 237-245.
- Hanel H. and Watson J. A. L. (1983) Preliminary field tests on the use of *Meteariizium anisopliae* for the control of *Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae). *Bull. Entomol. Res.* 73, 305-313.
- Hussey N. W. and Tinsley T. W. (1981) Impressions of insect pathology in the People's Republic of China. In *Microbial Control of Pests and Plant Diseases, 1970-1980* (Edited by Burges H. D.), pp. 783-795. Academic Press, London, New York.
- Ignoffo C. M. and Garcia C. (1985) Host spectrum and relative virulence of an Ecuadorian and Mississippian biotype of *Namuracea rileyi*. *J. Invertebr. Pathol.* 45, 346-352.
- Ignoffo C. M., Hostetter D. L., Blever K. D., Garcin G., Thomas G. D., Dickerson W. A. and Pianell R. (1978) Evaluation of an entomopathogenic bacterium, fungus and virus for control of *Heliothis zea* on soybeans. *J. Econ. Entomol.* 71, 163-168.
- Ignoffo C. M., Puglisi B., Hostetter D. L. and Dickerson W. A. (1976) Susceptibility of the cabbage looper, *Trichoplusia ni* and the velvet bean caterpillar, *Anticarsia gemmatalis* to several isolates of the entomopathogenic fungus *Namuracea rileyi*. *J. Invertebr. Pathol.* 28, 259-262.
- Jayarej S. (1985) Biological suppression of insect pests through the use of entomopathogens. In *Integrated Pest and Disease Management* (Edited by Jayarej S.), pp. 319-336 TNAU, Coimbatore, India.
- Jayarej S. (1986) Role of insect pathogens in plant protection. *Proc. Indian Natn. Sci. Acad.* 52, 91-107.
- Keller S. (1986) Quantitative ecological evaluation of the May beetle pathogen *Beauveria brongniartii* and its practical application. In *Fundamental and Applied Aspects of Invertebrate Pathology* (Edited by Samson R. A., Vial J. M. and Peters D.), pp. 178-181. Foundation of the Fourth Int. Colloq. Invertebr. Pathol., Wageningen.
- Keller S. and Zimmermann (1989) Mycopathogens of soil insects. In *Insect-Fungus Interactions* (Edited by Wilding N., Collins N. M., Hammond P. M. and Webber J. F.) pp. 240-270, Academic Press, London, New York.
- Kmitowa K., Bajer C. and Wojciechowski M. (1977) Differences in the pathogenicity of entomopathogenic fungi from France and Poland. *Polish Ecol. Studies* 3, 115-260.
- Roberts D. W. and Yendol W. G. (1971) Use of fungi for microbial control of insects. In *Microbial Control of Insects and Mites* (Edited by Burges H. D. and Hussey N. W.) pp. 123-149. Academic Press, London, New York.
- Rombach M. C., Aguda R. M., Shepard B. M. and Roberts D. W. (1986) Infestation of rice brown planthopper *Nilaparvata lugens* (Homoptera: Delphacidae) by field application of entomopathogenic hyphomycetes (Deuteromycotina). *Environ. Entomol.* 15, 1070-1073.
- Sarmasi A. (1969) Apparent infections of queens and drones of the mound building termite *Odonotermes obtusus* by *Aspergillus flavus*. *J. Invertebr. Pathol.* 10, 434-439.
- Silberg A. L. and Bezzecchi T. M. (1972) Toxic properties of *Beauveria bassiana* (Bals.) Vuille strains from insects. *Vopr. Zool. Razv.* 17, 68-74.
- Walsted J. D., Anderson R. F. and Sunbury W. J. (1970) Effects of environmental conditions on two species of entomopathogenic fungi, *Beauveria bassiana* and *Meteariizium anisopliae*. *J. Invertebr. Pathol.* 16, 220-226.
- Wood S. P. and Grula E. A. (1986) Utilizable surface nutrients on *Heliothis zea* available for growth of *Beauveria bassiana*. *J. Invertebr. Pathol.* 43, 259-269.